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Comparison of Pigeon Guillemot, *Cepphus columba*, Blood Parameters from Oiled and Unoiled Areas of Alaska Eight Years After the *Exxon Valdez* Oil Spill

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In 1997, we compared the haematological and plasma biochemical profiles among populations of pigeon guillemots, *Cepphus columba*, in areas oiled and not oiled by the 1989 *Exxon Valdez* oil spill (EVOS) that occurred in Prince William Sound (PWS), Alaska. Pigeon guillemot populations in PWS were injured by EVOS and have not returned to pre-spill levels. If oil contamination is limiting recovery of pigeon guillemots in PWS, then we expected that blood parameters of pigeon guillemots would differ between oiled and unoiled areas and that these differences would be consistent with either toxic responses or lower fitness. We collected blood samples from chicks at approximately 20 and 30 days after hatching. Physiological changes associated with chick growth were noted in several blood parameters. We found that only calcium and mean cell volume were significantly different between the chicks in oiled and unoiled areas. Despite these differences, blood biomarkers provided little evidence of continuing oil injury to pigeon guillemot chicks, eight years after the EVOS. Preliminary data from adults indicated elevated aspartate aminotransferase activity in the adults from the oiled area, which is consistent with hepatocellular injury. Because adults have greater opportunities for exposure to residual oil than nestlings, we recommend studies that fully evaluate the health of adults residing in oiled areas.

Keywords: alcid; *Cepphus columba*; *Exxon Valdez*; haematology; oil spill; pigeon guillemot; plasma biochemistry; Prince William Sound.

Introduction

Population estimates of pigeon guillemots, *Cepphus columba*, in Prince William Sound (PWS), Alaska, have declined from 15 000 individuals in 1972–73 to approximately 3 000 individuals in the mid-1990s (Dwyer *et al.*, 1976; Klosiewski and Laing, 1994; Agler and Kendall, 1997; Sanger and Cody, 1994). A large-scale regime in the Gulf of Alaska during the late 1970s (Piatt and Anderson, 1996) likely caused much of this decline, as high quality forage fish were more widely available in the 1970s than in recent years (Hayes and Kuletz, 1997; Kuletz *et al.*, 1997). Pigeon guillemot populations in PWS were further impacted by the Exxon Valdez oil spill (EVOS; Murphy *et al.*, 1997), when the supertanker *Exxon Valdez* ran aground on 24 March 1989 and spilled 42 million L of crude oil into PWS. Approximately 40% of this oil was deposited on the shorelines of PWS (Galt *et al.*, 1991). Between 100 000 to 375 000 birds died in the spill, of which 1500 to 3000 were pigeon guillemots (Piatt *et al.*, 1990). Seven years after the spill, pigeon guillemots had not recovered to pre-spill numbers (Agler and Kendall, 1997; Oakley and Kuletz, 1996). It is not clear to what extent demography, food availability or the physiological effects of lingering oil

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exposure may be constraining recovery of pigeon guillemots in PWS.

Pigeon guillemots are vulnerable to oil spills because they use the near-shore habitat (King and Sanger, 1979; Piatt *et al*., 1990). They breed in small colonies along rocky coastlines, and roost on intertidal rocks. Guillemots spend much of their time on the sea surface or diving for surface schooling fish, demersal fish and invertebrates associated with the intertidal and subtidal zones.

The prey of pigeon guillemots are also susceptible to oil contamination. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal habitats (Murphy *et al*., 1999; Rice, 1999). For example, Pacific herring, *Clupea pallasii*, embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan *et al*., 1996; Hose *et al*., 1996). Biomarkers of oil ingestion were noted in PWS fish several years after EVOS. Walleye pollack, *Theragra chalcogramma*, collected from oiled Naked Island in 1990 and 1991, exhibited high levels of fluorescent aromatic compounds in their bile (Collier *et al*., 1996). Jewett *et al*. (1995) reported that demersal fish in the oiled eelgrass beds of Herring Bay, PWS, demonstrated a high incidence of haemosiderosis lesions in the liver. Kelp greenling, *Hexagrammos decogrammus*, collected in 1996 showed significantly higher expression of P450 activity in oiled Herring Bay versus unoiled Jackpot Bay (Holland-Bartels *et al*., 1998). Research in the early 1990s demonstrated that oil exposure had detrimental effects on near-shore predators including river otters, *Lutra canadensis* (Bowyer *et al*., 1994; 1995, Duffy *et al*., 1993, 1994) and sea otters, *Enhydra lutris* (Loughlin *et al*., 1996). Whether residual oil from the EVOS affected pigeon guillemots required further evaluation.

Acute toxic effects of petroleum hydrocarbons are well known (Leighton, 1993), but the lingering effects of chronic oil exposure have not been investigated fully in free ranging piscivorous birds (Fry and Lowenstine, 1985). Leighton (1993) provided an extensive review of avian studies of petroleum oil toxicity. Dosing experiments have shown that the effects of oil ingestion include: (1) lower hatch rate and altered yolk structure (Grau *et al*., 1977; Szaro *et al*. 1978a); (2) reduced rate of growth (Szaro *et al*., 1978b; Peakall *et al*., 1982); (3) slower development and reduced survivorship of chicks (Trivelpiece *et al*., 1984); (4) liver, kidney and intestine damage in long-term exposure (Khan and Ryan, 1991; Patton and Dieter, 1980; Fry and Lowenstine, 1985); and (5) Heinz-body haemolytic anaemia associated with a substantial decrease in packed-cell volume (Leighton *et al*., 1983).

Because guillemot chicks remain in their natal burrow until they fledge, oil contamination can occur through contact with the oiled feathers of an adult while in the egg or chick stage, or through ingestion of contaminated fish (Leighton, 1993; Peakall *et al*., 1980). At nine days of incubation, avian embryos are extremely sensitive to oil contacting the egg shell. As little as 5 µl of Prudhoe Bay crude oil has been reported to cause embryo death (Albers, 1977; Szaro *et al*., 1978a). Dosing studies of weathered crude oil on congeneric black guillemots, *Cepphus grylle*, suggest that oil ingestion may cause long-term physiological effects which could reduce a young bird's ability to survive at sea (Peakall *et al*., 1980).

Payne *et al*. (1986) suggested that detecting simple changes in a biochemical or physiological response in a population may provide information on the presence of toxins. Haematological analyses (differential cell counts) may provide information about the immunological status of birds (Campbell, 1986a). Levels of plasma enzymes provide information on the function of organs, e.g. liver (Campbell, 1986a). Elevated levels of acute-phase protein haptoglobin indicate responses to exogenous toxins, bacterial or viral infections, and physical trauma (Silverman and LeGrys, 1987). Physiological changes occurring during the chick growth period have been suggested by many authors to influence blood parameters (Wolf *et al*., 1985; Hoffman *et al*., 1985; Kostlecka-Myrcha, 1987; Starck, 1998; Work, 1996; Prichard *et al*., 1997). To prevent age-dependent variation from biasing assessments, haematological and plasma biochemical profiles should be repeated on chicks at different stages of development.

To make an accurate assessment of clinical tests, reference values of healthy individuals are needed (Hawkey and Samour, 1988), but information on haematological and clinical chemistry on pigeon guillemots or other alcids is limited (Newman *et al*., 1997; Newman and Zinkl, 1998; Prichard *et al*., 1997; Kostleck-Myrcha, 1987). We assume therefore that colonies in the unoiled areas represent healthy populations. If oil contamination is limiting recovery of pigeon guillemots in PWS, we expected that blood chemistry and cell counts would differ between oiled and unoiled areas and these differences should be consistent with either toxic responses or lower fitness. In this study, we compare the haematological and plasma biochemical profiles between pigeon guillemot populations in an oiled area of PWS and in unoiled areas of PWS.

Methods and Materials

During summer 1997, measurements of growth and blood samples from pigeon guillemot chicks were collected in areas oiled by the EVOS and in reference areas that were not oiled (Fig. 1). The oiled area we evaluated was Naked Island (60° 40′ N, 147° 28′ W) in central PWS. The prevailing winds and currents during spring of 1989 deposited oil predominately on the east and north-west shorelines of Naked Island (Galt *et al*., 1991; Oakley and Kuletz, 1996). The combined colonies of Jackpot Island (60° 19′ N, 148° 11′ W) and Icy Bay (60° 14′ N, 148° 17′ W) in south-western PWS were not oiled and represent the reference areas in this study. For

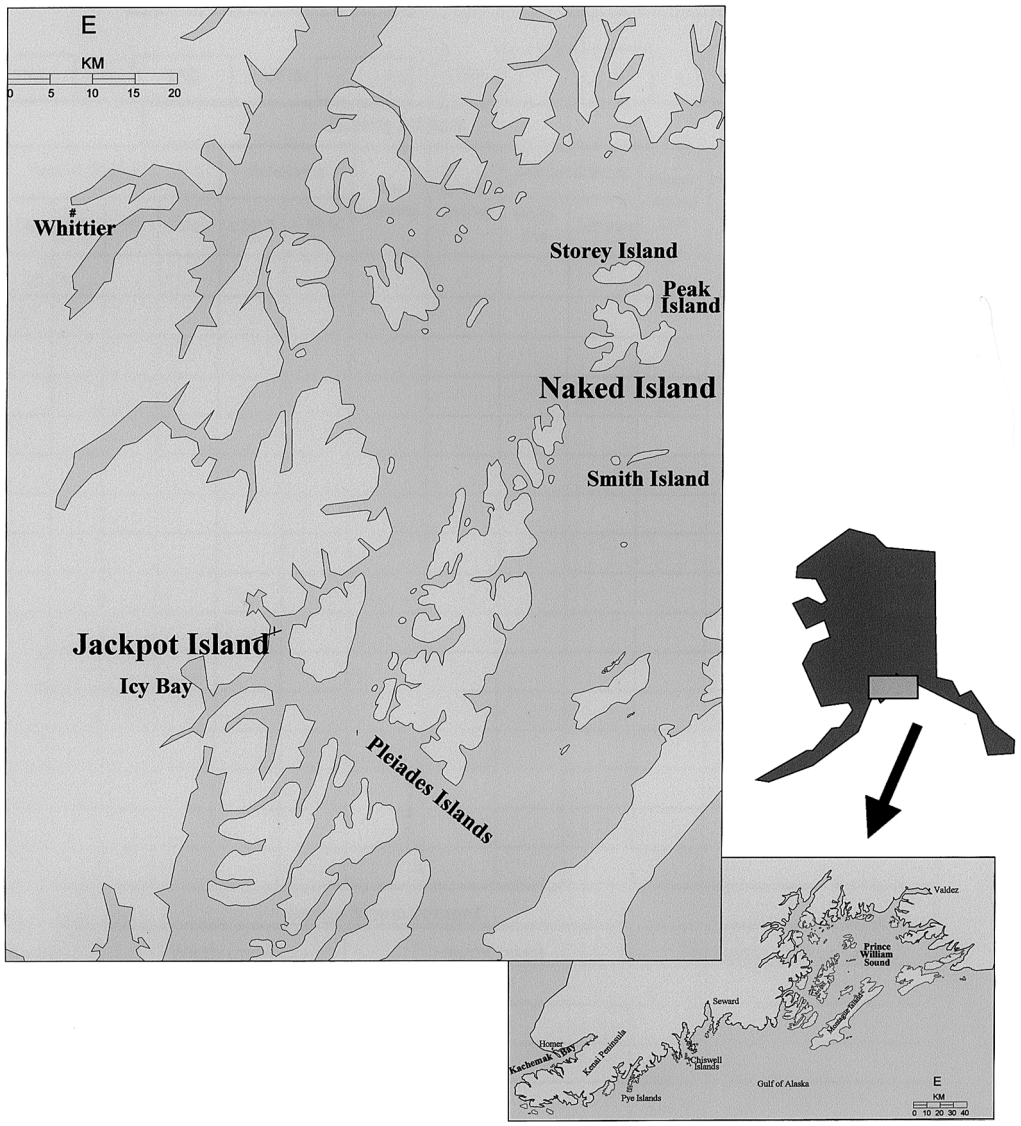

Fig. 1 Location of the Jackpot Island and Naked Island study areas in Prince William Sound and location of Prince William Sound within Southcentral Alaska.

evaluating adults, we also included a third reference area located in Kachemak Bay (59° 35′ N, 151° 19′ W), which is located in lower Cook Inlet, Alaska.

For each chick, mass and length of wing-chord were measured every five days until the chick fledged. When possible, two blood samples were collected from each chick at approximately 20 and 30 days after hatch. The hatching date of the chick was determined from either direct observation or was estimated by comparing wing-chord length for chicks of unknown age to wing-chord length for chicks of known age. Adults were captured either by noose traps placed on roosting rocks or with a dip net.

1 cc of blood was collected from the brachial vein of chicks using a one cc tuberculin syringe with a 25 or 26 gauge needle. Adults were bled from the medial metatarsal vein. Fresh blood was used to make blood smears on glass slides. Two heparinized micro-haematocrit tubes were filled with blood from the puncture site, capped with clay and stored in coolers. Whole blood was placed in microtainer tubes treated with lithium heparin. These samples were centrifuged within 2 h of collection. After centrifuging, plasma was removed with a disposable pipette and divided between two snap-top plastic vials. Vials were frozen in propane freezers. Blood smear slides, micro-haematocrit tubes and one vial of plasma were placed in chilled insulated boxes and shipped to the Avian and Exotic Laboratory of Redondo Beach, California within 48 h of collection. The following parameters were measured: red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), haemoglobin (Hb), mean cell haemoglobin content (MCHC), counts of white blood cells (WBC), heterophils, lymphocytes, eosinophils, basophils, activity of creatine phosphokinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST),

alkaline phosphatase, gamma-glutamyl transferase (GGT), concentration of calcium, uric acid, plasma protein, total protein, alpha-1 macroglobulin, alpha-2 macroglobulin, beta globulin, gamma globulin, albumin, albumin to gamma globulin ratio, bile acid, phosphorus and sodium. A second vial of frozen plasma was sent to the University of Alaska Fairbanks for measurement of haptoglobin concentration with electrophoresis kits (Helena Laboratories, Beaumont Texas, USA). Plasma was applied to agarose gels and electrophoresed at 100 volts for 1 h. Agarose plates were then fixed with 7.5% trichloroacetic acid and stained with o-dianisidine to detect the Hp-haemoglobin complex. The Hp-haemoglobin complex was quantified by densitometry and results are reported in mg haemoglobin binding capacity per 100 ml of plasma (Duffy *et al.*, 1994). Enzyme immunoassay wipes were used to evaluate the presence of polyaromatic hydrocarbon molecules on the plumage of adults. The plumage of adults was wiped with one-ply section of 5 by 5 cm gauze pad saturated with isopropanol. The gauze pad was then placed in aluminium foil and frozen until analysis. Levels of phenathrene, pentacosane and hexacosane from the wipes were measured with the EnSysEnviro-Gard™ Polynuclear Aromatic Hydrocarbon test kit 70608, produced by Millpore Corporation (Bedford, Massachusetts, USA) or detected with gas chromatography-mass spectrometry (Duffy *et al.*, 1999).

Data were tested for normality and equal variance with the Kolmogorov–Smirnov test with Littiefors correction and with the Levene median test, respectively. To test the hypothesis that there was no difference between samples collected at 20 days of age and 30 days of age, we used the paired *t*-test or the Friedmans test on ranks, a non-parametric test for a repeated measures design, on the samples collected in the reference area. Blood parameters with significantly different values between sampling ages are considered to be influenced by the development stage of the chicks. A *t*-test or Mann–Whitney test was used, when appropriate, to detect differences in blood parameters between oiled and unoiled areas, and between 30-day post hatch chicks and adults in the reference areas.

Results

Effects of age: nestlings

We found several age-related differences in the blood samples. For chicks in south-western PWS, significant differences between the blood samples of chicks 20 and 30 days after hatching included PCV ($P = 0.014$), RBC ($P = 0.002$) and alkaline phosphatase activity ($P = 0.001$). Differences in phosphorus concentrations were marginally non-significant ($P = 0.063$). The mean ($\pm$SD) wing-chord lengths of the 20-day and 30-day age groups were 92.8 ± 7.6 cm and 128.7 ± 6.3 cm, respectively. A multiple logistic regression model using variables RBC, PCV, and alkaline phosphatase activity correctly predicts the age group in 18 of 22 blood samples with a concordance of 82% (likelihood ratio test = 7.7, $P = 0.051$). Variables correlated with the wing-chord length of chicks included PCV ($r = 0.59$, $P = 0.001$, $n = 26$), RBC ($r = 0.58$, $P = 0.001$, $n = 24$), alkaline phosphatase ($r = 0.57$, $P = 0.003$, $n = 24$), phosphorus ($r = -0.39$, $P = 0.059$, $n = 24$)and Hb($r = 0.56$, $P = 0.004$, $n = 24$).

Effects of age: adults versus nestlings

The blood profiles of the adult birds from reference areas of Jackpot Island, Icy Bay and Kachemak Bay were distinct from the blood profile of the chicks from the reference area of Jackpot and Icy Bay. The age-related differences among chicks, which included PCV, RBC, alkaline phosphatase, and phosphorus, extended to our comparison between adults versus chicks. By the time a chick fledges, which occurs between 33 and 54 days of age, its weight is comparable to that of an adult, but its wing growth is not complete (Ewins, 1992, 1993). For adults from south-western PWS, the mean ($\pm$SD) for wing-chord length and body weight were 184 ± 4 cm and 508 ± 50 g, respectively. The wing-chord length at 20 and 30 days after hatching was 49% and 70% respectively, of wing-chord length in adults. The body mass at 20 and 30 days after hatching is 66% and 86%, respectively, of the adult body mass. Because we had only samples from four adults in south-western PWS, we incorporated adults from Kachemak Bay ($n = 3$) into our sample of adults from unoiled areas. In the unoiled areas, adults had higher PCV ($P = 0.001$), RBC ($P = 0.003$), Hb (0.004), AST ($P = 0.010$), and albumin concentrations ($P = 0.011$), and lower alkaline phosphatase ($P < 0.001$) and lower phosphorus concentrations ($P < 0.001$) than 30-day old chicks in south-western PWS. Adults also tended to have lower WBC ($P = 0.072$), calcium concentration ($P = 0.063$), and bile acid concentration ($P = 0.094$) than chicks.

Oiled vs. unoiled populations: nestlings

In the 20-day age group, chicks sampled from the oiled population at Naked Island had lower calcium ($P = 0.002$), plasma protein ($P = 0.008$), and alkaline phosphatase activity ($P = 0.025$), and a higher lymphocyte count ($P = 0.006$) than chicks in the unoiled area of south-western PWS (Table 1). In the 30-day age group, Naked Island had significantly lower calcium ($P = 0.043$) and MCV ($P = 0.015$) than chicks from south-western PWS (Table 2).

Oiled vs. unoiled populations: adults

Our sample size of adults was small. The number of adult blood samples from Naked Island, south-western PWS and Kachemak Bay were 10, 4 and 3, respectively. Adults at Naked Island were captured between 29 July and 3 August. Three of the adults in the reference areas were captured in June and two in August. Adults

TABLE 1

Mean, standard deviation (SD) and sample size (n) of the haematological and plasma chemistry of pigeon guillemot chicks sampled in 1997 at oiled Naked Island and unoiled Jackpot Island and Icy Bay, in Prince William Sound, Alaska (the estimated age of the chicks is 20 days).

	Oiled area			Unoiled area		
	Naked Island			Jackpot Island and Icy Bay		
	Mean	SD	n	Mean	SD	n
Red blood cells (mm^{-3})	2.6	0.4	14	2.58	0.4	17
Packed cell volume (%)	44	4	15	43	6	18
Mean cell volume (mm^{-3})	159	16	14	160	13	17
Haemoglobin (g dl^{-1})	12.7	1.6	14	11.6	1.6	16
MCHC (g dl^{-1})	30.7	5.4	14	28	4.3	16
White blood cells (10^3 mm^{-3})	13	5	14	16	6	18
Heterophil*	49	12	14	61	10	17
Lymphocytes *	49	12	14	37	10	17
Eosinophil	0.6	1.3	14	0.7	1.3	16
Basophil	1.4	1.3	14	1.1	1.4	18
Calcium (mg dl^{-1})*	8.9	1.9	14	11.0	1.2	18
CK (u l^{-1})	530	233	14	776	541	17
LDH (u l^{-1})	937	234	14	897	471	18
AST (u l^{-1})	277	106	14	221	119	16
Uric Acid (mg dl^{-1})	18.3	8.7	14	20.0	11.2	16
Plasma Protein (g dl^{-1})*	3.1	0.5	14	3.8	0.6	18
Total Protein (g dl^{-1})	4.5	0.6	14	4.8	0.8	18
Alpha−1 (g dl^{-1})	0.39	0.11	14	0.44	0.18	18
Alpha-2 (g dl^{-1})	0.70	0.31	14	0.75	0.32	18
Beta (g dl^{-1})	0.88	0.21	14	0.91	0.35	18
Gamma Globulin (g dl^{-1})	0.70	0.16	14	0.75	0.15	18
Albumin (g dl^{-1})	1.86	0.33	14	1.94	0.51	18
Albumin/Gamma Globulin (g dl^{-1})	0.72	0.17	14	0.68	0.15	18
Bile Acid Assay (umol l^{-1})	38.8	35.6	14	61.9	105	14
Alkaline phosphatase (u l^{-1})*	372	151	14	279	82	17
GGT (u l^{-1})	25.2	12.5	14	20.6	14.8	13
Phosphorus (mg dl^{-1})	9.6	4.8	13	6.2	1.7	17
Sodium (mmol l^{-1})	129	17	11	141.0	5	13
Haptoglobin (Hg binding dl^{-1})	109	40	15	124	51	16

* Means significantly different between chicks sampled at Naked Island and Jackpot–Icy Bay ($P < 0.050$).

captured in the oiled area had significantly higher AST activity ($P = 0.017$), lower RBC ($P = 0.006$), Hb ($P = 0.004$) *and GGT* ($P = 0.015$) than adults in the reference areas (Table 3). The AST activity for the adults in the oiled area was nearly double the levels for the adults in the reference areas. The plumage wipes from adults at Naked Island ($n = 10$) indicated low levels of phenathrene, pentacosane and hexacosane (mean $\pm$ SD : 0.004 ppm $\pm$ 0.002, 0.178 ppm $\pm$ 0.059, and 0.202 ppm $\pm$ 0.047, respectively).

Discussion

The clinical haematology and biochemistry of seabirds is not as well known as for waterfowl, poultry or pet species (Newman and Zinkl, 1998). Blood parameters vary among species according to life history patterns, diet, and activity level. Pigeon guillemots differ from more commonly studied birds in that they have rapidly growing semi-precocial chicks, their diet is composed of marine fish, and they are adapted to diving to depths greater than 20 m (Ewins, 1993). Interpreting our results is also made difficult because of the paucity of biochemical studies on this species. The few reference values for this species are from studies with sample sizes of less than ten individuals (Newman and Zinkl, 1998; Newman *et al*., 1997; Prichard *et al*., 1997; Haggblom *et al*., 1988; Bradley and Trefall, 1974). Our study extends the biochemical information for chicks of this species by providing reference values for different stages of development that are based on larger sample size.

Effects of development

Physiological changes occurring during post-hatch development of chicks affect many haematological and biochemical parameters (Starck, 1998; Vinuela *et al*., 1991; Kostlecka-Myrcha, 1987). Age-related variation in blood parameters is an important consideration when collecting samples from pigeon guillemot colonies, because the range in chick ages may be as great as 42 days (Drent, 1965). This is caused by asynchronous nesting and the laying of replacement clutches (Ewins, 1993; Drent, 1965). It has been well documented in many avian species that adults have higher PCV, RBC, and Hb than immature birds (Work, 1996; Wolf *et al*., 1985; Kostlecka-Myrcha, 1987; Fairbrother *et al*., 1990), but there is little documentation of the changes in these parameters within the nestling period for free-living species (Kostlecka-Myrcha, 1987). Anaemia has been associated with oil contamination (Hartung and Hunt,

TABLE 2

Mean, standard deviation (SD) and sample size (*n*) of the haematological and plasma chemistry of pigeon guillemot chicks sampled in 1997 at oiled Naked Island and unoiled Jackpot Island and Icy Bay, in Prince William Sound, Alaska (the estimated age of the chicks is 30 days).

	Oiled area			Unoiled area		
	Naked Island			Jackpot Island and Icy Bay		
	Mean	SD	*n*	Mean	SD	*n*
Red blood cells (mm^{-3})	3.16	0.40	24	2.95	0.42	13
Packed cell volume (%)	48	4	25	47	6	15
Mean cell volume (mm^{-3})*	148	13	24	160	10	13
Haemoglobin (g dl^{-1})	13.8	1.6	22	13	1.8	14
MCHC (g dl^{-1})	29	5	22	27	4	14
White blood cells (10^3 mm^{-3})	13	6	24	12	5	17
Heterophil	62	11	24	56	12	17
Lymphocytes	36	11	24	42	12	17
Eosinophil	0.3	0.5	24	0.2	0.4	17
Basophil	1.1	1.0	24	1.3	1.8	17
Calcium (mg dl^{-1})*	9.0	1.8	19	10.3	1.3	15
CK (u l^{-1})	613	528	20	554	221	15
LDH (u l^{-1})	863	482	21	863	325	15
AST (u l^{-1})	313	169	19	304	233	15
Uric acid (mg dl^{-1})	12.3	11.1	21	16.7	8.7	14
Plasma protein (g dl^{-1})	3.5	0.8	22	4.0	1.5	17
Total protein (g dl^{-1})	5.0	1.7	22	4.6	0.9	15
Alpha-1 (g dl^{-1})	0.50	0.37	22	0.40	0.19	15
Alpha-2 (g dl^{-1})	0.68	0.40	22	0.72	0.39	15
Beta (g dl^{-1})	0.98	0.35	22	0.90	0.49	15
Gamma globulin (g dl^{-1})	0.75	0.38	22	0.73	0.19	15
Albumin (g dl^{-1})	0.75	0.17	22	0.70	0.21	15
Albumin/gamma globulin (g dl^{-1})	2.14	0.75	22	1.84	0.50	15
Bile acid assay (umol l^{-1})	38	45	15	106	158	14
Alkaline phosphatase (u l^{-1})	502	367	18	443	152	15
GGT (u l^{-1})	16	15	14	16	11	13
Phosphorus (mg dl^{-1})	7.4	4.5	21	5.6	1.9	15
Sodium (mmol l^{-1})	133	16	16	142	13	13
Haptoglobin (Hg binding dl^{-1})	99	38	20	122	44	14

* Means significantly different between chicks sampled at Naked Island and Jackpot–Icy Bay ($P < 0.050$).

1966; Szaro *et al*., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Leighton *et al*., 1983). Clinical signs of anaemia are low PCV, RBC, MCV or MCHC. Therefore it was critical for us to identify these age-specific differences in red blood cell parameters before evaluating the health of immature birds. During the nestling period, there are dramatic changes in the profile of the red blood cells as embryonic forms, natal forms and adult forms replace one another (Schenk *et al*., 1978). Kostlecka-Myrcha (1987) documented PCV increases and MCV decreases during the nestling period of the little auk, *Plautus alle*, as smaller sized adult red blood cells replace the red blood cells after hatching. The greatest increases in RBC occurred during the first ten days post-hatch (Kostlecka-Myrcha, 1987; Hoffman *et al*., 1985). Post-hatch development of erythropoietic tissue is closely related to growth of body mass. As the chick approaches adult size or asymptotic body mass, bones are ossifying in preparation for flight and erythropoietic tissue decreases to adult levels (Starck, 1998). Pigeon guillemot chicks reach asymptotic growth between 30 and 40 days of age (Ewins, 1993). Kostlecka-Myrcha (1987) noted a non-significant increase in Hb level during the latter half of nestling period. Our study and the study of Haggblom *et al*. (1988) confirm that similar age-related changes in Hb occur in pigeon guillemots chicks. We expect subtle changes in red blood cells and Hb to continue after chicks fledge.

Elevated alkaline phosphatase (AP) activity in birds is associated with increased osteoblastic activity such as skeletal growth and repair, egg production, or nutritional deficiencies (Lumeij, 1994). Therefore the normal range of AP activity in rapidly growing chicks is higher than in adults (Wolf *et al*., 1985; Hoffman *et al*., 1985; Vinuela *et al*., 1991; Work, 1996). We found AP activity nearly doubled between the samples for chick 20 and 30 days after hatching. The activity of AP reported by Newman and Zinkl (1998) for fledglings were similar to the AP activity for 20-day old chicks in our study. In red kites, *Milvus milvus*, Vinuela *et al*. (1991) reported that AP activity peaked at 38 days after hatch, when the growth of long bones were near completion. Pigeon guillemot chicks also had higher phosphorus and marginally higher calcium levels than adults. Vinuela *et al*. (1991) noted that increases in calcium and phosphorus levels correlated with increases in AP activity during the nestling period of red kites. In brown pelicans, *Pelecanus occidentalis*, Wolf *et al*. (1985) found that AP activity and phosphorus concentration were highest during the first 10 months of development and remained

TABLE 3

Mean, standard deviation (SD) and sample size (*n*) of the haematological and plasma chemistry of adult pigeon guillemots sampled in 1997 from oiled Naked Island, Prince William Sound and unoiled areas of Jackpot Island/Icy Bay, Prince William Sound and Kachemak Bay, Lower Cook Inlet in Alaska.

	Oiled area			Unoiled area		
	Naked Island			Jackpot Island, Icy Bay and Kachemak Bay		
	Mean	SD	*n*	Mean	SD	*n*
Red blood cells (mm^{-3})*	3.01	0.35	10	3.76	0.59	6
Packed cell volume (%)	53	5	10	58	6	7
Mean cell volume (mm^{-3})	168	9	10	163	10	6
Haemoglobin (g dl^{-1})	18.3	3.3	10			
MCHC (g dl^{-1})	34.3	7.12	10	33.2	11.4	4
White blood cells (10^3 mm^{-3})	8	2	10	8	1	7
Heterophil	58	13	10	64	13	7
Lymphocytes	37.9	8.8	10	33.4	12.1	7
Eosinophil	0	0	10	0	1	7
Basophil	4	5	10	3	2	7
Calcium (mg dl^{-1})	8.6	1.6	9	9.1	1.2	7
CK (u l^{-1})	244	168	9	375	339	7
LDH (u l^{-1})	892	296	10	915	143	7
AST (u l^{-1})*	979	816	10	461	199	7
Uric acid (mg dl^{-1})	14.85	5.83	10	14.6	6.5	7
Plasma protein (g dl^{-1})	4.7	2.3	10	3.9	0.7	6
Total protein (g dl^{-1})	5.5	0.98	10	5.6	1.7	7
Alpha-1 (g dl^{-1})	0.45	0.24	10	0.43	0.29	7
Alpha-2 (g dl^{-1})	0.67	0.42	10	0.90	0.45	7
Beta (g dl^{-1})	0.90	0.58	10	0.71	0.30	7
Gamma globulin (g dl^{-1})	0.69	0.17	10	1.02	0.97	7
Albumin (g dl^{-1})	2.75	0.71	10	2.63	0.71	7
Albumin/gamma globulin (g dl^{-1})	1.03	0.30	10	0.94	0.27	7
Bile acid assay (umol l^{-1})	40.3	74.5	7	2.05	2.6	7
Alkaline phosphatase (u l^{-1})	93	70	8	137	102	6
GGT (u l^{-1}) *	3	5	9	10.8	8.2	7
Phosphorus (mg dl^{-1})	2.2	1.8	8	1.7	0.8	7
Sodium (mmol l^{-1})	138.6	17.1	7	143.8	9.3	4
Haptoglobin (Hg binding dl^{-1})	122	28	8	93	50	7

* Means significantly different between adults sampled at Naked Island and Jackpot–Icy-Kachemak Bays ($P < 0.050$).

moderately elevated through the first two years of life. Guillemots are smaller than pelicans, but their skeletal growth continues after fledging for at least two months (Ewins, 1992). These patterns suggest that AP activity, phosphorus and calcium concentrations of guillemot chicks will peak prior to fledging then gradually drop to adult range within the first six months of life.

Elevated WBC is a symptom of infection. Interpretation of elevated WBC in juvenile birds is difficult because their normal range is variable and higher than adults (Fudge, 1996). For terns, shearwaters and petrels, Work (1996) reported that older chicks tend to have higher WBC than adults. Puerta *et al.* (1990) reported similar results for common cranes. We could not detect differences in WBC between 20-day and 30-day old chicks, but these chicks had higher WBC than adults.

Similar to our results, Prichard *et al.* (1997) and Work (1996) reported that chicks had lower AST activity than adults. Newman and Zinkl (1998) found that young pigeon guillemots between five and 10 weeks old have AST activity greater than or equal to the activity in adults. Elevated AST activity is associated with hepatocellular damage, septicemia and muscle injury. Bollinger *et al.* (1989) studied the effect of different capture methods on waterfowl AST activity and reported that AST activity becomes elevated with physical exertion. We suggest that chicks have lower AST activity than adults because they are sedentary and their muscles are less developed. Compared to adults, chicks are little resistance to capture and are less likely to experience muscular exertion and injury.

Age-related differences in Hp concentration have been documented in mammals. Stellar sea lion, *Eumetopias jubatus*, pups that are less than 15 days old have significantly lower haptoglobin (Hp) levels than adults (Zenteno-Savin *et al.*, 1997). In humans, neonates do not have detectable levels of Hp until two months of age (Henry, 1991). Prichard and co-workers (1997) reported that pigeon guillemot chicks had significantly lower Hp levels than adults. Adults in our study had lower mean Hp levels than reported by Prichard (1997), which may explain why we did not find similar age-related differences. Prichard (1997) noted that Hp was correlated with the rate at which adults deliver meals to the nest. In our study Hp was significantly correlated with the rate of weight gain immediately prior to the drawing of blood from chicks. This relationship supports Prichard's speculation that Hp is sensitive to the nutrition of

chicks. We also documented a positive correlation between Hp and RBC, which suggests that Hp levels may be linked to the development of erythropoietic tissue during chick development.

Comparison between populations in oiled and unoiled areas

Various oil-dosing studies have been conducted on birds, but the symptoms of toxicity of oil ingestion have varied with species, age, the chemical composition of the oil, the dosing levels and the presence of additional stress factors (Hartung, 1995; Leighton, 1993). Ingestion of sublethal levels of crude oil may constitute a non-specific stressor for birds and render them more vulnerable to stress factors such as persistent cold temperatures and bacterial diseases (Holmes *et al*., 1979). To evaluate the presence of injury at the oiled colonies in this study, we measured blood parameters that were indicators of physiological health of organ systems that involve the liver function, kidney function, the haematopoietic system, immune function and electrolyte balance.

The avian liver responds to oil ingestion with hypotrophic activity (Szaro *et al*., 1978b; Patton and Dieter, 1980; Stubblefield *et al*., 1995) and induction of hepatic cytochrome P-450 (Peakall *et al*., 1989; Lee *et al*., 1985). Enlargement of the liver may be a compensatory response to metabolize the high burden of toxic material introduced in experimental diets (Patton and Dieter, 1980; Stubblefield *et al*., 1995) or an inflammation response to cell injury. Hepatocellular damage and necrosis are associated with elevation in the activity of plasma liver enzymes (Lewandowski *et al*., 1986). In Leighton's (1993) review of oil toxicity research, he found that the evidence of injury to the liver was inconsistent among studies, which may be associated with enzyme responses that are specific to species (Franson *et al*., 1985). Our indicators of liver injury were elevated bile acid, AST and LDH activity in the plasma. In pigeons, *Columba livia*, elevated levels of bile acid (Lumeij, 1988) and AST are the most sensitive indicator of experimentally induced liver injury (Lumeij, 1988; Campbell, 1986b). Ingestion stimulates the release of bile acid. Fasted peregrine falcons experienced a three-fold increase in plasma bile acid concentration after ingestion of meat (Lumeij and Remple, 1992). During our study, adults feed their nestlings at rate of 0.4–1.0 fish h^{-1}. We did not control the food intake of chicks and this would explain some of variation in bile acid concentrations between individuals. Post-prandial increases in bile acid concentration represent one-fold to two-fold increases, while hepatobilary disease results in a five-fold to 10-fold increases relative to the reference range (Lumeij, 1991). Elevated levels of bile acid concentration (exceeding 200 μmol l^{-1}) indicate persistent loss of hepatic function (Fudge, 1996). The bile acid concentrations of chicks at Naked Island were in the ranges reported for pigeons and peregrine falcons (Lumeij, 1988; Lumeij and Remple, 1992). While AST and LDH are considered non-specific because they occur in many tissues, Campbell (1986b) found that AST and LDH were sensitive indicators of liver disease in carnivorous birds including red tail hawks, *Buteo jamicensis*, and great horned owls, *Bubo virginianus*. Elevated BA, AST or LDH concentrations were uncommon among chicks in both the oiled and unoiled areas, and we did not observe a significant difference in mean activity of BA, AST or LDH between chicks of Naked Island and south-western PWS. Other researchers working with weathered Prudhoe Bay crude oil found no effect of oil dosing on liver enzyme responses of alcid chicks (Leighton, 1993; Prichard, 1997) and mallards (Rattner, 1981; Stubblefield *et al*., 1995). The blood variables associated with liver function and hepatocellular damage do not indicate deleterious effects on livers of chicks at Naked Island.

Renal tubular necrosis was documented in Cassin's auklets, *Prychoramphus aleuticus*, after oil was applied to their breast feathers (Fry and Lowenstine, 1985). Increases in uric acid in the plasma may indicate adverse effects on renal function (Allen, 1988; Fudge, 1996). In veterinary practices uric acid level values greater than 20 mg dl^{-1} are abnormal (Allen, 1988; Fudge, 1996). Newman and coworkers (1997) noted that uric acid levels in adult piscivorous marine birds are typically higher than in other avian species. They suggest that high protein diets combined with the osmoregulation demands of living in a marine environment cause higher concentrations of serum uric acid. In our study, both chicks and adults had uric acid levels that were below 20 mg dl^{-1}, which is within the reference range previously reported for adult pigeon guillemots (Newman and Zinkl, 1998; Newman *et al*., 1997). Therefore, the uric acid levels of chicks in the oiled area of our study does not appear to indicate the presence of impaired renal function or damage.

Anaemia was documented in several species of birds following exposure to oil (Hartung and Hunt, 1966; Szaro *et al*., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Fry and Addiego, 1987; Leighton *et al*., 1983). Reduced PCV and Heinz-body haemolytic anaemia was documented in young herring gulls, *Larus argentatus,* and Atlantic puffins, *Fratercula arctica*, after experimental ingestion of crude oil (Leighton *et al*., 1983). Yet, ingestion of high doses of Prudhoe Bay crude oil did not result in anaemia in both adult rhinoceros auklets, *Cerrorhinca monocerata* (Newman, personal communication) and mallards (Stubblefield *et al*., 1995). Haemolytic anaemia was documented in adult white-winged scoters, *Melanitta fusca*, rescued from an oil spill, but blood samples were taken several days after the birds were captured (Yamato *et al*., 1996). The decrease in physical activity, the stress of handling and the change in diet associated with captivity may influence erythropoiesis in adult alcids (Newman, personal communication). Anaemia is the result of reduced erythropoiesis, accelerated erythrocyte destruction

(haemolytic anaemia), or blood loss. Clinical signs of anaemia are low PCV, RBC, MCHC or MCV. There is little variation in PCV among species, and values below 32% are considered diagnostic of anaemia (Hawkey and Samour, 1988). In our study, the values for PCV, MCHC and haemoglobin were within the ranges that are normal for immature birds, which indicates that there was probably no anaemia for chicks in the oiled area of our study. The MCV values for 30-day old chicks at Naked Island were significantly less than the MCV for chicks in south-western PWS and in Kachemak Bay, Alaska (Seiser, unpublished data). It is not clear why MCV values are lower in the oiled area.

Immunosuppression has been noted in various oil dosing studies (Leighton, 1993). Reduced lymphocytes and reduced resistance to bacterial pathogens have been recorded in mallards (Holmes *et al*., 1979; Rocke *et al*., 1984). In adult rhinoceros auklets, ingestion of crude oil elicited no inflammatory response in WBC or differential cell counts, but young alcids may respond differently (Newman, personal communication). Leighton (1986) reported morphological changes to the lymphoid glands of young Atlantic puffins and herring gulls. In our study, WBC and differential cell counts (lymphocytes, heterophils, eosinophils and basophil) were our indicators of the state of the immune system. The ratio of lymphocytes to heterophils for the 20-day old chicks at Naked Island was significantly different from the ratio for chicks in south-western PWS, but this pattern did not persist for the 30-day old chicks. We found that Naked Island did not have significantly lower values of WBC or differential cell counts than the unoiled area in south-western PWS, which suggests that the immune system was not stressed or impaired in a way that would influence cell production.

Hypertrophy of salt glands has been documented in marine birds dosed with crude oil (Peakall *et al*., 1980, 1982, 1983; Miller *et al*., 1978). Osmoregulatory impairment can be accompanied by increases in plasma sodium levels. Peakall *et al*. (1980) noted a transient rise in plasma sodium levels in black guillemot chicks dosed with 0.1 and 0.2 ml of Prudhoe Bay crude oil. Similar results have been found in herring gulls (Miller *et al*., 1978) and mallards (Eastin and Rattner, 1982). In contrast, Prichard (1997) found that sodium levels of pigeon guillemot chicks did not respond to dosing with 0.2 ml weathered Prudhoe Bay crude oil. The sodium levels for chicks in the unoiled area of our study were similar to levels for the contol chicks in the study by Prichard *et al*. (1997). Because the sodium levels for the chicks at Naked Island were not significantly different from the levels for chicks in south-western PWS, we conclude that there is no evidence for hypertrophy of salt glands.

The results reported here also extend the database for Hp levels in pigeon guillemots. Haptoglobin is an acute phase protein that has been widely used in human and other mammal medical practices as an indicator of inflammatory diseases, infectious diseases, trauma or stress. Gevaert and co-workers (1991) demonstrated that Hp concentrations increased after the pigeons were infected with salmonellosis. Although Hp has been employed to assess potential stressors in compromised wildlife populations (Duffy *et al*., 1993, 1994; Zenteno-Savin *et al*., 1997; Prichard *et al*. 1997), it has not been widely used for assessing health in free-ranging birds. The recovery of river otters from the initial impact of the EVOS was documented in river otters with the use of Hp (Duffy *et al*., 1993, 1994). In comparisons between declining and stable populations of pinnipeds, significantly higher Hp concentrations were associated with the declining populations of harbour seals, *Phoca vitulina*, and sea lions (Zenteno-Savin *et al*., 1997). Prichard *et al*. (1997) examined the use Hp as a potential biomarker of oil ingestion in pigeon guillemot chicks, but found variation in growth rates and feeding rates among chicks from different colonies that confounded their interpretation of Hp response to the ingestion of weathered crude oil. In our study, there was no evidence of poor health identified by our suite of health indicators, which is consistent with the similar Hp levels we observed in chicks from oiled and unoiled areas.

Because nearly all the chicks that were sampled for blood in our study ultimately fledged, we conclude that our handling and blood sampling did not affect survival. This observation also supports our diagnosis of clinically healthy chicks. In contrast, the overall fledging success (fledglings per hatchling) for Naked Island and Jackpot Island was 46% and 68%, respectively. In Kachemak Bay, Prichard (1997) also noted that the majority of nestling mortality occurred in the first 12 days after hatch. Predators or food shortages are the most common sources of mortality of young chicks (Hayes and Kuletz, 1997; Nelson, 1987). Mink, a major predator of nestlings in PWS, was not present on Jackpot Island in 1997, but was at Naked Island. The shoreline of Naked Island suffered both oil contamination and physical disturbance from efforts to clean beaches after the spill. Both events tend to have negative effects on the prey base of pigeon guillemots. Therefore, we limit our conclusions on the health of chicks to the latter half of the nesting period. Currently, hematological and biochemical variables of the pigeon guillemots we studied provide little evidence of oil-related injury for chicks that hatched in 1997, eight years after the Exxon Valdez oil spill. In contrast to chicks, the pilot study we conducted on adult health suggests that the issue of oil-related injury in pigeon guillemot adults cannot be dismissed without further study.

Pigeon guillemot adults have greater opportunities for exposure to oil than nestlings. Adults feed on invertebrates including crabs, shrimps and bivalves (Oakley, 1981; Kuletz, 1983; Sanger, 1987), but rarely provision their chicks with invertebrates (Oakley, 1981; Ewin, 1993). In the winter, invertebrate consumption may increase because of seasonal changes in distribution of prey fish. Pacific sand lance, *Ammodytes hexapterus*, are

inaccessible because they are burrowed in the sediment, and young cod move to deeper waters (Oakley, 1981; Sanger, 1987). Bioaccumulation of polynuclear aromatic hydrocarbons (PAH) is greater in invertebrates than fish. Invertebrates cannot metabolize PAH as efficiently as fish, because of differences in the activity of mixed function oxgenenase enzymes and metabolic rate between invertebrates and fish (Gibson, 1977; Hellou, 1996). Therefore, adults potentially have a greater dietary source of PAHs than nestlings (Bolger *et al*., 1996; Baumard *et al*., 1998).

It is important to recognize that our sample of adults is small and was obtained opportunistically. The majority of the samples from the unoiled areas were obtained in June, while the samples from the oiled area were collected in late July and early August. Also, we do not know the sex of the birds we sampled. Sex and reproductive condition have been documented to affect plasma biochemistry (Wolf *et al*., 1985; Fairbrother *et al*., 1990; Gee *et al*., 1981). Because interpretation of differences between blood parameters for adults from the oiled and unoiled areas in our study is complicated by sampling issues, the interpretation we present is preliminary and should be viewed with some caution.

In comparison to adults in the unoiled area of our study, GGT activity was significantly lower for adults in the oiled area. GGT activity is commonly measured in mammal clinical practices to detect cholestatic diseases of the liver or the consumption of drugs and other toxic substances that induce the microsomal enzyme system (Henry, 1991). For example, fungi infested feed produces elevated plasma GGT activity in domestic chickens (Espada *et al*., 1994). GGT activity is not a sensitive indicator of avian hepatocellular injury (Campbell, 1986b). Egg laying also appears to elevate serum GGT activity. In domestic mallard hens, Fairbrother *et al*. (1990) observed that serum GGT activity was 10-fold higher during the egg-laying period compared to the incubation period. Newman and Zinkl (1998) measured the mean serum GGT activity for several seabird species, and reported a mean GGT activity of 16.5 IU l^{-1} with a range of 0–60 IU l^{-1} for five pigeon guillemot adults captured during the egg laying period. These values were slightly higher than the values we observed for adults in the unoiled areas of our study, which were also sampled early in the breeding season. For adults at Naked Island, which were sampled late in the breeding season, the GGT activity was within the range previously reported for adult rhinoceros auklets, *C. monocerata*, common murre, *Uria aalge*, incubating western gull, *L. occidentalis*, and non-breeding white pelicans, *P. onocrotalus* (Newman and Zinkl, 1998; Puerta *et al*., 1991). It is not clear if the lower GGT activity we observed for adults in the oiled area represents a normal seasonal trend in GGT activity for adult pigeon guillemots.

The AST activity of adults in the oiled area was significantly higher and nearly double the AST activity of adults in the unoiled areas of our study and double the AST activity of adult pigeon guillemots observed in other studies (Newman *et al*., 1997; Newman and Zinkl, 1998). Elevated AST activity is associated with both hepatocellular damage and muscle injury (Bollinger *et al*., 1989). Muscle injury associated with capture causes elevated CK or LDH activity in waterfowl species (Bollinger *et al*., 1989; Franson *et al*., 1985; Fudge, 1996). We did not observe significant differences in CK or LDH between adults in oiled and unoiled areas of our study. Because similar capture methods were used in the oiled and unoiled areas of our study, we suggest that the elevated AST concentrations in the adults from the oiled area are more consistent with hepatocellular injury than muscle injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Because adults have greater opportunities for exposure to residual oil than nestlings, we recommend additional studies to fully evaluate the health of adults residing in oiled areas.

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